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THERAPEUTIC METHOD

FIELD OF THE INVENTION

5 This application claims priority from Australian provisional patent application No. PS1606, filed on 8 April 2002.

This invention relates to the use of an antagonist of a G protein-coupled receptor in the prevention and/or treatment of fibrosis, such as the treatment of fibrosis

10 associated with myocardial infarction, diabetes, or certain pulmonary conditions. In a preferred embodiment the antagonist is a C5a receptor antagonist, more preferably a cyclic peptide antagonist of the C5a receptor.

15 ## BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the

20 references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does

25 not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

G protein-coupled receptors are prevalent throughout the human body, comprising approximately 60% of

30 known cellular receptor types. They mediate signal transduction across the cell membrane for a very wide range of endogenous ligands and consequently participate in a diverse array of physiological and pathophysiological processes, including, but not limited to, those associated

35 with cardiovascular, central and peripheral nervous system reproductive, metabolic, digestive, immunoinflammatory, and growth disorders, as well as other cell regulatory and proliferative disorders. Agents which selectively modulate

functions of G prot:ein-coupled receptcs have the potential
for therapeutic app)lications. These rceptors are becoming
increasingly recognised as important drug targets, due to
their crucial roles in signal transduction (G protein-
5   coupled receptors, IBC Biomedical Library Series, 1996)

One of the most intensively studied G protein-
coupled receptors is the receptor for C5a. C5a is one of
the most potent chemotactic agents known, recruiting
neutrophils and macrophages to sites of injury, altering
10   their morphology; inducing degranulation; increasing
calcium mobilisation, vascular permeability (oedema) and
neutrophil adhesiveness; contracting smooth muscle;
stimulating the release of inflammatory mediators,
including histamine, TNF-α, IL-1, IL-6, IL-8,
15   prostaglandins, and leukotrienes, and of lysosomal enzymes;
promoting the formation of oxygen radicals; and enhancing
antibody production (Gerard and Gerard, 1994).

Overexpression or underregulation of C5a is
implicated in the pathogenesis of immune system-mediated
20   inflammatory conditions, such as rheumatoid arthritis,
adult respiratory distress syndrome (ARDS), systemic lupus
erythematosus, tissue graft rejection, ischaemic heart
disease, reperfusion injury, septic shock, psoriasis,
gingivitis, atherosclerosis, Alzheimer's disease, lung
25   injury and extracorporeal post-dialysis syndrome, and in a
variety of other conditions (Whaley 1987; Sim 1993).

Agents which limit the pro-inflammatory actions of
C5a have potential for inhibiting chronic inflammation, and
its accompanying pain and tissue damage. For these
30   reasons, molecules which prevent C5a from binding to its
receptors are useful for treating chronic inflammatory
disorders driven by complement activation. Such compounds
also provide valuable new insights into the mechanisms of
complement-mediated immunity.
35   In our previous applications No. PCT/AU98/00490
and Australian provisional No. PR8334, the entire

disclosures of whic h are incorporated erein by this
reference, we descr ibed the three-dimesional structure of
some analogues of the C-terminus of human C5a, and used
this information to design novel compounds which bind to
5   the human C5a receptor (C5aR), behaving as either agonists
or antagonists of C5a. It had previously been thought that
a putative antagonist might require both a C-terminal
arginine and a C-terminal carboxylate for receptor binding
and antagonist activity (Konteatis et al, 1994). In
10   PCT/AU98/00490 we showed that in fact a terminal
carboxylate group is not generally required either for high
affinity binding to C5aR or for antagonist activity.
Instead we found that a hitherto unrecognised structural
feature, a turn conformation, was the key recognition
15   feature for high affinity binding to the human C5a receptor
on neutrophils. As described in our Australian provisional
application No. PR8334, filed on 17th October 2001, we used
these findings to design constrained structural templates
which enable hydrophobic groups to be assembled into a
20   hydrophobic array for interaction with a C5a receptor. We
have subsequently found that preferred compounds of this
class are able to inhibit inflammatory bowel disease,
osteoarthritis, and hypersensitivity states, and this is
described in our Australian provisional applications
25   No. 2002952084, filed on 16th October 2002, No. 2002952086,
filed on 16th October 2002, and No. 2002952129, filed on 17th
October 2002 respectively. The entire disclosures of these
specifications are incorporated herein by this reference.
         Fibrosis, the ingrowth of fibroblasts and the
30   production of extracellular matrix to form abnormal
scarring, can result from many causes, including trauma,
surgical interventions, infections and pathological
conditions. Fibrosis is a sequel of conditions such as
chronic inflammation, including inflammation arising from
35   diabetes and hypertension, but can arise in the absence of
inflammation. It can occur in a variety of tissues,

including but not 1.imited to the lung, kidney, liver and
heart. Fibrosis cc ntributes to the los of function
experienced in such conditions, through the formation of
abnormal quantities of extracellular matrix which change
5   the physical properties of the scarred tissue. Diabetes-
or hypertension-induced fibrosis of the heart, for
instance, produces stiffening of the ventricle walls which
contributes to decreased cardiac output.

It is estimated that 45% percent of deaths in the
10   USA are attributable to disorders exhibiting proliferative
fibrosis. Although fibrosis is debilitating and may be
life-threatening, and the number of individuals who may
benefit from an effective antifibrotic therapy is large,
currently there are no effective treatments available.
15   Both acute and chronic diseases which induce inflammation
in the lung can lead to an irreversible process
characterized by pulmonary fibrosis (PF). Pulmonary
fibrosis may also occur as a side-effect of treatment with
chemotherapeutic agents such as bleomycin. Pulmonary
20   fibrosis is a severe disease, which leads to functional
impairment and death. Cardiac fibrosis is associated with
chronic hypertension, and both cardiac and renal fibrosis
are long-term sequelae of diabetes.

Fibrosis is a dynamic process, and is considered
25   to be potentially reversible. The extracellular matrix
laid down during fibrosis may be resorbed after the
withdrawal of the fibrotic stimuli. In many cases,
however, the presence of fibrosis is only identified after
loss of function has already taken place, for instance
30   where decreased cardiac output is a sign of otherwise
undetected cardiac fibrosis. Consequently, while it is
desirable in certain circumstances to be able to prevent
fibrosis from occurring, it is also desirable to be able to
reverse existing fibrosis once it is detected. However,
35   current therapeutic options for the treatment of fibrotic
conditions are limited and relatively ineffective (el-Nahas

et al. 1997).

The effects of drug-induced and hypertension-induced pulmonary and renal fibrosis in animal models can be prevented or partially reversed by compounds which act

5 by suppressing inflammatory events and down-regulating lung pro-collagen I over-expression (Iyer et al., 1999a,b).

We have shown that the administration of pirfenidone or spironolactone can prevent and partially reverse cardiac fibrosis and the increase in cardiac

10 stiffness which occurs in streptozotocin-induced diabetes in rats (Miric G, et al., 2001) It is thought that pirfenidone acts by inhibiting increased TGF-β mRNA expression, allowing an increase in expression of metalloproteases which degrade the collagen I laid down

15 during fibrosis. The mode of action of spironolactone is at present unknown. Spironolactone is a steroid analogue which is primarily used as a diuretic; pirfenidone (5-methyl-1-phenyl-2-(1H)-pyridone), an investigational compound being investigated as an anti-fibrotic agent in a

20 number of indications.

It would be highly desirable to identify other therapeutically or prophylactically active agents for use in the treatment or prevention of fibrosis.

25 SUMMARY OF THE INVENTION

The overexpression or underregulation of a G-protein-coupled receptor, the C5a receptor, has been implicated in immune-system mediated events such as inflammation. Agents which influence C5a receptor

30 activity, such as C5a receptor antagonists, have the potential to mediate inflammatory events, and may provide a means of therapeutic or prophylactic intervention, but have not previously been suggested as potential agents in the treatment or prevention of fibrosis.

35 We have now surprisingly found that a cyclic peptide with C5a receptor antagonist has the ability to

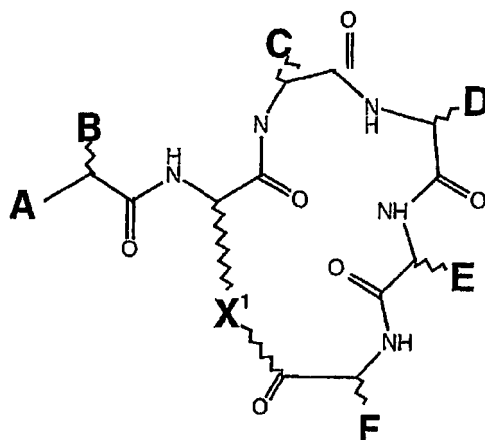ameliorate cardiac fibrosis in an animal model of this condition.

According to a first aspect, the invention provides a method of prevention, treatment or alleviation
5   of a fibrotic condition, comprising the step of administering an effective amount of an antagonist of a G protein-coupled receptor to a subject in need of such treatment.

The use of any compound having activity as an
10 antagonist of a G protein-coupled receptor, and particularly as a C5a receptor antagonist, is contemplated, including but not limited to those disclosed in our earlier International patent applications No.PCT/AU98/00490 or No. PCT/AU02/01427 or in International patent applications No.
15 PCT/US00/11187 by Neurogen Corporation and No. PCT/JP01/06902 by Welfide Corporation, or antibody antagonists such as those disclosed in PCT/US00/24219 or US patent No. 6355245. The entire disclosures of all of these specifications are incorporated herein by this cross-
20 reference.

More preferably the C5a receptor antagonist is a peptide or a peptidometic compound, and more preferably is a cyclic peptide or a cyclic peptidometic compound. Even more preferably the compound is a cyclic peptide or a
25 cyclic peptidometic compound of PCT/AU98/00490 or PCT/AU02/01427.

Still more preferably the antagonist is a compound which

(a)   is an antagonist of a G protein-coupled receptor,
30 (b)   has substantially no agonist activity, and
(c)   is a cyclic peptide or peptidomimetic compound of formula I

where A is H, alkyl, aryl, NH_2, NH-alkyl,
$N(alkyl)_2$, NH-aryl, NH-acyl, NH-benzoyl, $NHSO_3$, $NHSO_2$-alkyl,
$NHSO_2$-aryl, OH, O-alkyl, or O-aryl;

       B is an alkyl, aryl, phenyl, benzyl, naphthyl or
indole group, or the side chain of a D- or L-amino acid
such as L-phenylalanine or L-phenylglycine, but is not the
side chain of glycine, D-phenylalanine, L-
homophenylalanine, L-tryptophan, L-homotryptophan, L-
tyrosine, or L-homotyrosine;

       C is a small substituent, such as the side chain
of a D-, L- or homo-amino acid such as glycine, alanine,
leucine, valine, proline, hydroxyproline, or thioproline,
but is preferably not a bulky substituent such as
isoleucine, phenylalanine, or cyclohexylalanine;

       D is the side chain of a neutral D-amino acid such
as D-Leucine, D-homoleucine, D-cyclohexylalanine, D-
homocyclohexylalanine, D-valine, D-norleucine, D-homo-
norleucine, D-phenylalanine, D-tetrahydroisoquinoline, D-
glutamine, D-glutamate, or D-tyrosine, but is preferably
not a small substituent such as the side chain of glycine
or D-alanine, a bulky planar side chain such as D-
tryptophan, or a bulky charged side chain such as D-
arginine or D-Lysine;

E is a bulky substituent, suh as the side chain
of an amino acid se lected from the grop consisting of L-
phenylalanine, L-tryptophan and L-homotryptophan, or is L-
1-napthyl or L-3-benzothienyl alanine, but is not the side

5   chain of D-tryptophan, L-N-methyltryptophan,
L-homophenylalanine, L-2-naphthyl L-tetrahydroisoquinoline,
L-cyclohexylalanine, D-leucine, L-fluorenylalanine, or
L-histidine;

F is the side chain of L-arginine, L-homoarginine,
10   L-citrulline, or L-canavanine, or a bioisostere thereof,
ie. a side chain in which the terminal guanidine or urea
group is retained, but the carbon backbone is replaced by a
group which has different structure but is such that the
side chain as a whole reacts with the target protein in the
15   same way as the parent group; and

X is $-(CH_2)_nNH-$ or $(CH_2)_n-S-$, where n is an integer
of from 1 to 4, preferably 2 or 3; $-(CH_2)_2O-$;
$-(CH_2)_3O-$; $-(CH_2)_3-$; $-(CH_2)_4-$; $-CH_2COCHRNH-$; or
$-CH_2-CHCOCHRNH-$, where R is the side chain of any common or
20   uncommon amino acid.

In C, both the *cis* and *trans* forms of
hydroxyproline and thioproline may be used.

Preferably A is an acetamide group, an aminomethyl
group, or a substituted or unsubstituted sulphonamide
25   group.

Preferably where A is a substituted sulphonamide,
the substituent is an alkyl chain of 1 to 6, preferably 1
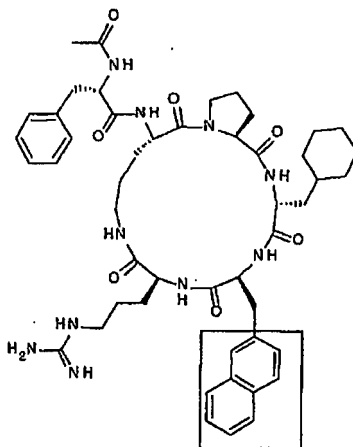to 4 carbon atoms, or a phenyl or toluyl group.

Preferably the antagonist is a C5a receptor
30   antagonist. In a particularly preferred embodiment, the
compound has antagonist activity against C5aR, and has no
C5a agonist activity.

The compound is preferably an antagonist of C5a
receptors on human and mammalian cells including, but not
35   limited to, human polymorphonuclear leukocytes and human
macrophages. The compound preferably binds potently and

selectively to C5a receptors, and morepreferably has
potent antagonist a ctivity at sub-micrmolar
concentrations. Even more preferably the compound has a
receptor affinity IC50<25µM, and an antagonist potency
5   IC50<1µM.

In particularly preferred embodiments the compound
is selected from the group consisting of compounds **1** to **6**,
10 to **15**, **17**, **19**, **20**, **22**, **25**, **26**, **28**, **30**, **31**, **33** to **37**, **39**
to **45**, **47** to **50**, **52** to **58** and **60** to **70** described in
10   International patent application No.PCT/AU02/01427. In a
particularly preferred embodiment, the compound is PMX53
(compound **1**), compound **33**, compound **60** or compound **45**
described therein.

Most preferably the compound is the compound
15   designated PMX53, disclosed in PCT/AU98/00490, which has
the formula

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30   In a second aspect, the invention provides the use
of a C5a receptor antagonist for the manufacture of a
medicament for use in the treatment of a fibrotic
condition.

For the purposes of this specification, the term
35   "C5a receptor antagonist" includes any compound which can
reduce or inhibit effects mediated by the interaction

between C5a and C5a ι receptor. Thus th term includes polyclonal or monoc :lonal antibodies, pptides, peptidomimetics, and non-peptide compounds.

5   Methods and pharmaceutical carriers for preparation of suitable formulations for administration by any desired route may be prepared by standard methods, for example by reference to well-known textbooks such as Remington: The Science and Practice of Pharmacy, Vol. II, 1995 (19th edition), A.R. Gennaro (ed), Mack Publishing

10   Company, Easton, Pennsylvania, or Australian Prescription Products Guide, Vol. 1, 1995 (24th edition) J. Thomas (ed), Australian Pharmaceutical Publishing Company Ltd, Victoria, Australia.

        The compounds may be administered at any suitable

15   dose and by any suitable route. Oral, transdermal or intranasal administration is preferred, because of the greater convenience and acceptability of these routes. The effective dose will depend on the nature of the condition to be treated, and the age, weight, and underlying state of

20   health of the individual being treated. This will be at the discretion of the attending physician or veterinarian. Suitable dosage levels may readily be determined by trial and error experimentation, using methods which are well known in the art.

25   The carrier or diluent, and other excipients, will depend on the route of administration, and again the person skilled in the art will readily be able to determine the most suitable formulation for each particular case.

        While it is particularly contemplated that the

30   subject for treatment by the method of the invention is human, the treatment is also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as felids, canids, bovids,

35   and ungulates.

BRIEF DESCRIPTION C)F THE FIGURES

Figure 1a shows a comparison of the daily water intake for control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean ± SEM. The arrow indicates initiation of L-NAME treatment.

Figure 1b shows a comparison of thedaily L-NAME intake for L-NAME and L-NAME+C5a receptor antagonist treated rats.

Figure 2 shows a comparison of the body weight of control rats and rats treated with control agent + C5a antagonist, L-NAME and L-NAME+C5a antagonist. Values are expressed as mean ± SEM. The arrow indicates initiation of L-NAME treatment.

Figure 3 shows a comparison of systolic blood pressure measurements of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats at day 32. Values expressed as mean ± SEM. *p< 0.05 compared to control. **p< 0.05 compared to L-NAME.

Figure 4 shows a comparison of the left ventricular wet weight of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values expressed as mean ± SEM. *p< 0.05 compared to control.

Figure 5 shows a comparison of the interstitial collagen deposition in the left ventricle of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean ± SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

(a)   interstitial

(b)   perivascular

Figure 6a shows a comparison of interstitial collagen deposition in the heart ventricles of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean ± SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

(a)   left vent ricle

(b)   right ven tricle

        Figure 7 shows a comparison of collagen
deposition in the kidneys of control, control+C5a
antagonist, L-NAME and L-NAME+C5a antagonist treated rats.
Values are expressed as mean ± SEM. *p <0.05 compared to
control; ** p <0.05 compared to L-NAME.

(a)   tubulointerstitial

(b)   glomerular

        Figure 8 shows a comparison of inflammatory cell
count in the heart ventricles of control, control+C5a
antagonist, L-NAME and L-NAME+C5a antagonist treated rats.
Values are expressed as mean ± SEM. *p <0.05 compared to
control; ** p <0.05 compared to L-NAME.

(a)   left ventricle

(b)   right ventricle

        Figure 9 summarizes echocardiographic data for
control, control+C5a antagonist, L-NAME and L-NAME+C5a
receptor antagonist treated rats. *p<0.05 compared to
control; **p<0.05 compared to L-NAME.

a.   Left ventricular wall thickness in diastole.

b.   Left ventricular internal diameter in diastole.

c.   E/A flow ratio.

d.   Diastolic volume.

e.   Cardiac output.

        Figure 10 shows a comparison of diastolic
stiffness constants for control, control+C5a antagonist, L-
NAME and L-NAME+C5a antagonist treated rats. Values
expressed as mean ± SEM. *p <0.05 compared to control;
**p<0.05 compared to L-NAME.

        Figure 11 shows a comparison of developed
pressure for control, control+C5a antagonist, L-NAME and L-
NAME+C5a antagonist treated rats. Values are expressed as
mean ± SEM.

        Figure 12 shows haematoxylin and eosin-
stained sections of rat lung at x40 magnification.

a) N ormal lung.

b) Lung 7-9 days after intra-tracheal
bleomycin instillation, showing severe patchy lesions
around the airways. There was no significant difference
5   between PMX53-treated rats and non-treated rats (n=4 in
each group).

Figure 13a shows a higher magnification view
of a patchy lesion (x200) showing inflammatory cells in the
alveolar space and alveolar septa, with leakage of red
10 cells and plasma. Figure 13b shows a higher magnification
view of normal lung (x200), showing the two types of
alveolar epithelial cells (AECs): type I AECs (40%) are
flat cells, and form 90% of the surface lining of the
alveolar sacs and alveoli (double arrows). Type II AECs
15 (60%) are rounded cells which are commonly located in
obtuse angles in the polygonal alveolus (arrows) rather
than the surface region. When the alveolar epithelium is
exposed to certain toxic agents, particularly if there is
extensive destruction of the sensitive type I AECs, type II
20 AECs increase in size and number.

Figure 14 shows the increased size and number
of Type II alveolar epithelial cells in lungs of bleomycin-
treated rats (arrows) (x200).

Figure 15 shows the effect of PMX53 on
25 bleomycin-induced collagen deposition in acute lung
inflammation (7-9 days).

Figure 16 shows lung tissue from a bleomycin-
instilled, PMX53-treated rat 18 days after instillation,
illustrating the decrease in size of patchy lesions around
30 the airways compared to the acute inflammatory stage
illustrated in Figure 12b (x40).

Figure 17 shows a higher magnification view
(x200) of lung tissue from a non-drug treated bleomycin-
instilled rat.
35   a) Alveolar macrophages (arrows) in the
alveolar space.

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b) Increase in alveolarwall thickness, with some collagen deposition (arrow) in th alveolar septa.

Figure 18 shows collagen as detected by Picro Sirius Red staining in rat lung (x40).

a) Normal rat.

b) Non-treated bleomycin instilled rat, showing increased collagen in thickened alveolar wall.

c) Non-drug treated bleomycin instilled rat, showing typical fibrous foci in the alveolar space.

Figure 19 shows the effect of PMX53 on bleomycin-induced collagen deposition in rat lung at 18 days after bleomycin instillation.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this specification, the term "fibrotic condition" is to be taken to mean any fibrotic disorder, such as multiple sclerosis, retinal disorders including proliferative vitroretinopathy and macular degeneration, scleroderma, sclerosing peritonitis, fibrosis arising from trauma, burns, chemotherapy, radiation, infection or surgery and fibrosis of major organs such as the kidney, liver, heart or lungs.

The term "C5a receptor antagonist" includes any compound which can reduce or inhibit effects mediated by the interaction between C5a and C5a receptor. Thus the term includes polyclonal or monoclonal antibodies, peptides, peptidomimetics, and non-peptide compounds.

The terms "treating," "treatment," and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

As used herein, the singular forms "a", "an", and

"the" include plura 1 reference unless he context clearly
dictates otherwise.   Thus, for example a reference to "an
enzyme" includes a plurality of such enzymes, and a
reference to "an amino acid" is a reference to one or more

5   amino acids.

Unless defined otherwise, all technical and
scientific terms used herein have the same meaning as
commonly understood by one of ordinary skill in the art to
which this invention belongs. Although any materials and

10 methods similar or equivalent to those described herein can
be used to practice or test the present invention, the
preferred materials and methods are now described.

For the purposes of this specification, the term
"alkyl" is to be taken to mean a straight, branched, or

15 cyclic, substituted or unsubstituted alkyl chain of 1 to 6,
preferably 1 to 4 carbons. Most preferably the alkyl group
is a methyl group. The term "acyl" is to be taken to mean
a substituted or unsubstituted acyl of 1 to 6, preferably 1
to 4 carbon atoms. Most preferably the acyl group is

20 acetyl. The term "aryl" is to be understood to mean a
substituted or unsubstituted homocyclic or heterocyclic
aryl group, in which the ring preferably has 5 or 6
members.

A "common" amino acid is a L-amino acid selected

25 from the group consisting of glycine, leucine, isoleucine,
valine, alanine, phenylalanine, tyrosine, tryptophan,
aspartate, asparagine, glutamate, glutamine, cysteine,
methionine, arginine, lysine, proline, serine, threonine
and histidine.

30 An "uncommon" amino acid includes, but is not
restricted to, D-amino acids, homo-amino acids, N-alkyl
amino acids, dehydroamino acids, aromatic amino acids other
than phenylalanine, tyrosine and tryptophan, ortho-, meta-
or para-aminobenzoic acid, ornithine, citrulline,

35 canavanine, norleucine, γ-glutamic acid, aminobutyric acid,

L-fluorenylalanine,   L-3-benzothienylalaine, and
α,α-disubstituted am lino acids.

Generally, the terms "treating", "treatment" and
the like are used herein to mean affecting a subject,
5   tissue or cell to obtain a desired pharmacological and/or
physiological effect. The effect may be prophylactic in
terms of completely or partially preventing a disease or
sign or symptom thereof, and/or may be therapeutic in terms
of a partial or complete cure of a disease.
10   "Treating" as used herein covers any treatment of,
or prevention of disease in a vertebrate, a mammal,
particularly a human, and includes: preventing the disease
from occurring in a subject who may be predisposed to the
disease, but has not yet been diagnosed as having it;
15   inhibiting the disease, ie., arresting its development; or
relieving or ameliorating the effects of the disease, ie.,
cause regression of the effects of the disease.

The invention includes the use of various
pharmaceutical compositions useful for ameliorating
20   disease. The pharmaceutical compositions according to one
embodiment of the invention are prepared by bringing a
compound of formula I, analogue, derivatives or salts
thereof and one or more pharmaceutically-active agents or
combinations of compound of formula I and one or more
25   pharmaceutically-active agents into a form suitable for
administration to a subject using carriers, excipients and
additives or auxiliaries.

Frequently used carriers or auxiliaries include
magnesium carbonate, titanium dioxide, lactose, mannitol
30   and other sugars, talc, milk protein, gelatin, starch,
vitamins, cellulose and its derivatives, animal and
vegetable oils, polyethylene glycols and solvents, such as
sterile water, alcohols, glycerol and polyhydric alcohols.
Intravenous vehicles include fluid and nutrient
35   replenishers. Preservatives include antimicrobial, anti-
oxidants, chelating agents and inert gases. Other

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pharmaceutically ac ceptable carriers iclude aqueous
solutions, non-toxi c excipients, including salts,
preservatives, buffers and the like, as described, for
instance, in Remington's Pharmaceutical Sciences, 20th ed.
5 Williams & Wilkins (2000) and The British National
Formulary 43rd ed. (British Medical Association and Royal
Pharmaceutical Society of Great Britain, 2002;
http://bnf.rhn.net), the contents of which are hereby
incorporated by reference. The pH and exact concentration
10 of the various components of the pharmaceutical composition
are adjusted according to routine skills in the art. See
Goodman and Gilman's The Pharmacological Basis for
Therapeutics (7th ed., 1985).
      The pharmaceutical compositions are preferably
15 prepared and administered in dosage units. Solid dosage
units include tablets, capsules and suppositories. For
treatment of a subject, depending on activity of the
compound, manner of administration, nature and severity of
the disorder, age and body weight of the subject, different
20 daily doses can be used. Under certain circumstances,
however, higher or lower daily doses may be appropriate.
The administration of the daily dose can be carried out
both by single administration in the form of an individual
dose unit or else several smaller dose units and also by
25 multiple administration of subdivided doses at specific
intervals.
      The pharmaceutical compositions according to the
invention may be administered locally or systemically in a
therapeutically effective dose. Amounts effective for this
30 use will, of course, depend on the severity of the disease
and the weight and general state of the subject.
Typically, dosages used *in vitro* may provide useful
guidance in the amounts useful for *in situ* administration
of the pharmaceutical composition, and animal models may be
35 used to determine effective dosages for treatment of the
cytotoxic side effects. Various considerations are

described, eg. in L anger, Science, 249 1527, (1990).
Formulations for or al use may be in th form of hard
gelatin capsules, in which the active ingredient is mixed
with an inert solid diluent, for example, calcium
5   carbonate, calcium phosphate or kaolin. They may also be
in the form of soft gelatin capsules, in which the active
ingredient is mixed with water or an oil medium, such as
peanut oil, liquid paraffin or olive oil.

Aqueous suspensions normally contain the active
10 materials in admixture with excipients suitable for the
manufacture of aqueous suspensions. Such excipients may be
suspending agents such as sodium carboxymethyl cellulose,
methyl cellulose, hydroxypropylmethylcellulose, sodium
alginate, polyvinylpyrrolidone, gum tragacanth and gum
15 acacia; dispersing or wetting agents, which may be (a) a
naturally occurring phosphatide such as lecithin; (b) a
condensation product of an alkylene oxide with a fatty
acid, for example, polyoxyethylene stearate; (c) a
condensation product of ethylene oxide with a long chain
20 aliphatic alcohol, for example, heptadecaethylenoxycetanol;
(d) a condensation product of ethylene oxide with a partial
ester derived from a fatty acid and hexitol such as
polyoxyethylene sorbitol monooleate, or (e) a condensation
product of ethylene oxide with a partial ester derived from
25 fatty acids and hexitol anhydrides, for example
polyoxyethylene sorbitan monooleate.

The pharmaceutical compositions may be in the form
.. of a sterile injectable aqueous or oleaginous suspension.
This suspension may be formulated according to known
30 methods using suitable dispersing or wetting agents and
suspending agents such as those mentioned above. The
sterile injectable preparation may also a sterile
injectable solution or suspension in a non-toxic
parenterally-acceptable diluent or solvent, for example, as
35 a solution in 1,3-butanediol. Among the acceptable
vehicles and solvents which may be employed are water,

Ringer's solution, and isotonic sodiumchloride solution.
In addition, steril e, fixed oils are cnventionally
employed as a solvent or suspending medium. For this
purpose, any bland fixed oil may be employed, including
5   synthetic mono-or diglycerides. In addition, fatty acids
such as oleic acid may be used in the preparation of
injectables.

Agents useful in the invention may also be
administered in the form of liposome delivery systems, such
10   as small unilamellar vesicles, large unilamellar vesicles,
and multilamellar vesicles. Liposomes can be formed from a
variety of phospholipids, such as cholesterol,
stearylamine, or phosphatidylcholines.

Dosage levels of the compounds of the present
15   invention will usually be of the order of about 0.5mg to
about 20mg per kilogram body weight, with a preferred
dosage range between about 0.5mg to about 10mg per kilogram
body weight per day (from about 0.5g to about 3g per
patient per day). The amount of active ingredient which
20   may be combined with the carrier materials to produce a
single dosage will vary, depending upon the host to be
treated and the particular mode of administration. For
example, a formulation intended for oral administration to
humans may contain about 5mg to 1g of an active compound
25   with an appropriate and convenient amount of carrier
material, which may vary from about 5 to 95 percent of the
total composition. Dosage unit forms will generally
contain between from about 5mg to 500mg of active
ingredient.
30   It will be understood, however, that the specific
dose level for any particular patient will depend upon a
variety of factors including the activity of the specific
compound employed, the age, body weight, general health,
sex, diet, time of administration, route of administration,
35   rate of excretion, drug combination and the severity of the
particular disease undergoing therapy.

In additi on, some of the compunds of the
invention may form solvates with wateror common organic
solvents. Such solvates are encompassed within the scope
of the invention.

5   The compounds of the invention may additionally be
combined with other therapeutic compounds to provide an
operative combination. It is intended to include any
chemically compatible combination of pharmaceutically-
active agents, as long as the combination does not

10 eliminate the activity of the compound of this invention.
For example, spironolactone, pirfenidone, *Gingko biloba*
extract (Welt et al, 1999), and tocopherol acetate (Rosen
et al, 1995) are known in the art for treatment of
fibrosis. Inhibitors of prolyl hydroxylase, procollagen C-

15 proteinase, also known as bone morphogenetic protein-1
(BMP-1), or connective tissue growth factor 02450, WO00/are
being investigated for this purpose by FibroGen, Inc. See
for example WO/01/56996, WO/01/15729, WO00/02450,
WO00/50390, WO00/27868, WO00/13706, and WO/9921860.These

20 compounds include prostacyclin and phenanthroline
derivatives. The invention includes within its scope
combinations of C5a inhibitors and such known agents.

General Methods

25

Peptide synthesis

        Cyclic peptide compounds of formula I are prepared
according to methods described in detail in our earlier
applications No. PCT/AU98/00490 and No. PCT/AU02/01427, the

30 entire disclosures of which are incorporated herein by this
reference. While the invention is specifically illustrated
with reference to the compound AcF-[OPdChaWR] (PMX53),
whose corresponding linear peptide is Ac-Phe-Orn-Pro-dCha-
Trp-Arg, it will be clearly understood that the invention

35 is not limited to this compound.
        Compounds **1-6, 17, 20, 28, 30, 31, 36** and **44**

disclosed in Intern ational patent applcation
No.PCT/AU98/00490 a nd compounds **10-12,14, 15, 25, 33, 35,
40, 45, 48, 52, 58, 60, 66,** and **68-70** disclosed for the
first time in International patent application
5   PCT/AU02/01427 have appreciable antagonist potency (IC50 <
1 μM) against the C5a receptor on human neutrophils. PMX53
and compounds **33, 45** and **60** of PCT/AU02/01427are most
preferred.

       We have found that all of the compounds of formula
10   I which have so far been tested have broadly similar
pharmacological activities, although the physicochemical
properties, potency, and bioavailability of the individual
compounds varies somewhat, depending on the specific
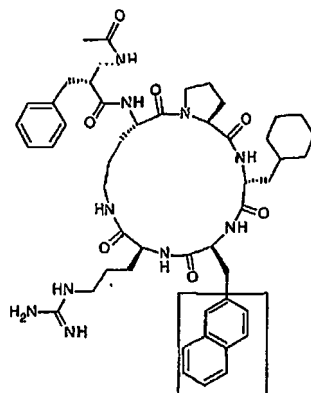substituents.

15   The general tests described in PCT/AU98/00490 and
PCT/AU02/01427 may be used for initial screening of
candidate inhibitor of G protein-coupled receptors, and
especially of C5a receptors.

       The invention will now be described in detail by
20   way of reference only to the following non-limiting
examples and figures.

       *Example 1   Effect of a C5a receptor antagonist on L-
                    NAME-induced cardiac fibrosis*

25

       Male Wistar rats (8 weeks old) were obtained from
the Central Animal Breeding House of The University of
Queensland. The rats were administered a C5a receptor
antagonist designated PMX53, which has the formula:

        This agent was administered at a dosage of
1mg/kg/day orally for 4 days before rats were additionally
treated with nitro-L-arginine methyl ester (L-NAME) for 4
weeks, ie a total duration of treatment of antagonist of 32
days. L-NAME administration produces hypertension and
cardiac remodelling as a result of inhibition of the
production of nitric oxide (NO).

        L-NAME was administered at a concentration of
400mg/l in the drinking water for 4 weeks to give a mean
daily intake of 18.7 ± 0.4mg L-NAME (41.4 ± 0.8mg/kg mean
body weight). Body weight and food and water intakes were
measured daily.

        Neither L-NAME nor C5a receptor antagonist
treatment altered water intake or growth rate, as shown in
Figures 1 and 2.

        Systolic blood pressure was measured in selected
unanaesthetised rats, using a tail-cuff method. As
illustrated in Figure 3, systolic blood pressure increased
from 118 ± 3mmHg to 160 ± 2mmHg in L-NAME-treated rats
without significantly altering heart rate or increasing
left ventricular weight, as determined by echocardiograph
or post-mortem examination, when compared to control rats.
These results are shown in Figure 4.

Similarly , right ventricularand other major organ
weights were not si gnificantly alteredwith L-NAME
treatment.

C5a receptor antagonist treatment of L-NAME rats
5   significantly increased systolic blood pressure by 16mmHg
to 176 ± 3mmHg, resulting in an increased left ventricular
wet weight. Additionally, C5a receptor antagonist
treatment of control rats induced a non-significant
increase in blood pressure. These results are summarised
10   in Figures 3 and 4. C5a receptor antagonist treatment of
both control and L-NAME rats did not significantly alter
wet weights of the remaining major organs.

After 4 weeks of L-NAME treatment, heart function
was determined *in vivo* by echocardiography and *in vitro*
15   using the isolated Langendorff heart preparation described
below. Collagen deposition was measured by image analysis
using laser confocal microscopy of picrosirius red-stained
cardiac slices, as described below.

Rats were euthanased with pentobarbitone (100
20   mg/kg ip). Blood was taken from the abdominal vena cava,
centrifuged and the plasma frozen. Plasma glucose was
measured by Precision Plus Blood Glucose Electrodes
(Medisense, Abbott Laboratories); plasma Na^+ and K^+ were
measured by flame photometry.
25

a)   Collagen distribution
Collagen distribution was determined by image
analysis of sections of heart and kidney stained with
picrosirius red (0.1% Sirius Red F3BA in picric acid),
30   which selectively stains fibrillar collagen. Slides were
left in 0.2% phosphomolybdic acid for 5 minutes, washed,
and left in picrosirius red for 90 minutes, then in 1 mM
HCl for 2 minutes and 70% ethanol for 45 seconds. The
stained sections were analyzed with an Image Pro plus
35   analysis program using an Olympus BH2 microscope, with
results expressed as a percentage of red area in each

screen. At least 4 areas were examine in each heart. The
results are present ed in Figures 5, 6 nd 7.

Image analysis showed an increase of 108% in
interstitial collagen and an 87% increase in perivascular
5   collagen in the left ventricle of L-NAME treated rats when
compared to controls. Similarly, a significant increase in
collagen levels was observed in the right ventricle, where
a 175% increase in interstitial and a 37% increase in
perivascular collagen content occurred. L-NAME treatment
10   also significantly increased the collagen content by 55% in
the tubulointerstitial areas of the kidneys with a smaller
increase in glomerular spaces.

C5a receptor antagonist treatment attenuated the
increased collagen deposition. In C5a antagonist treated
15   rats, L-NAME treatment produced 23% and 43% of the increase
observed in rats treated with L-NAME only when comparing
the left ventricular interstitial and perivascular areas
respectively. Similar results were observed in the right
ventricle, where C5a receptor antagonist treatment of L-
20   NAME restricted collagen deposition to 44 and 37% in the
interstitial and perivascular areas respectively. In the
kidneys, C5a antagonist administration to L-NAME rats
restricted collagen deposition to 30% in the interstitium
and normalized the increase in glomerular collagen
25   concentrations observed in L-NAME treated rats.

As illustrated in Figure 9, L-NAME treatment
resulted in a large inflammatory cell infiltration in both
the left and right ventricles. A 30-fold increase in
inflammatory cell population was observed in the both left
30   and right ventricular interstitial and perivascular areas
following L-NAME treatment. C5a receptor antagonist
treatment totally prevented inflammatory cell infiltration
into left or right ventricles following L-NAME treatment.
No information is so far available on inflammatory cell
35 · type or kidney infiltration.

b)   Echocardi ographic analysis

Cardiac f unction was estimatd *in vivo* using echocardiography, using conventional methods.

Although L-NAME treatment did not significantly
5   increase left ventricular weight, echocardiographic M-mode measurements showed that L-NAME treatment had triggered cardiac remodelling, increasing the left ventricular wall thickness and decreasing the left ventricular internal diameter in diastole. Further L-NAME treatment
10 significantly increased the ratio of early (E) to atrial (A) mitral valve inflow rates (E/A ratio), and significantly decreased diastolic volume and cardiac output. Fractional shortening and ascending aortic flow rates were not significantly altered by L-NAME treatment.
15 Thus L-NAME treatment induces cardiac remodelling, with minor changes in systolic function and an improved diastolic function.

C5a receptor antagonist treatment of control rats did not significantly alter any parameter measured by
20 echocardiographic analysis. C5a receptor antagonist treatment of L-NAME rats normalised the increase in left ventricular wall thickness and decreased left ventricular internal dimensions. This treatment also significantly normalised the E/A ratio, diastolic volume and cardiac
25 output. These results are presented in Figure 9.

c)   Isolated Langendorff heart preparation

The Langendorff isolated heart preparation was used to determine the diastolic stiffness of the left
30 ventricles *ex vivo*.

Rats were anaesthetised with sodium pentobarbitone (100mg/kg ip) and heparin (2000 IU) was administered via the femoral vein. After allowing 2 minutes for the heparin to fully circulate, the heart was excised and placed in
35 cooled (0°C) crystalloid perfusate (Krebs-Henseleit solution of the following composition in mM: NaCl 118, KCl 4.7, $MgSO_4$

1.2, KH$_2$PO$_4$ 1.2, CaC l_2 2.3, NaHCO$_3$ 25.0 glucose 11.0). The
heart was then atta .ched to the cannula with the tip of the
cannula positioned immediately above the coronary ostia of
the aortic stump, and perfused in a non-recirculating

5   Langendorff fashion at 100cm of hydrostatic pressure. The
buffer temperature was maintained at 35°C. The hearts were
punctured at the apex to facilitate thebesian drainage and
paced at 250 bpm.

A balloon catheter was inserted in the left

10 ventricle via the mitral orifice for measurement of left
ventricular developed pressure. The catheter was connected
via a three-way tap to a micrometer syringe and to a
Statham P23 pressure transducer. The outer diameter of the
catheter was similar to the mitral annulus to prevent

15 ejection of the balloon during the systolic phase. After a
10 minute stabilisation period, steady-state left
ventricular pressure was recorded from isovolumetrically
beating hearts. Increments in balloon volume were applied
to the heart until left ventricular end-diastolic pressure

20 reached approximately 30mmHg.

To assess myocardial stiffness in isolated
Langendorff hearts, stress (σ, dyne/cm2) and tangent
elastic modulus (E, dyne/cm2) for the midwall at the
equator of the left ventricle were calculated by assuming

25 spherical geometry of the ventricle and considering the
midwall equatorial region as representative of the
remaining myocardium:

$$\sigma = \frac{VP}{W}\left(1 + \frac{4(V+W)}{[V^{1/3} + (V+W)^{1/3}]^3}\right)$$

30

$$E = 3\left\{\frac{VP}{W} - \sigma + \frac{\left[\frac{\sigma}{V} + \frac{(W\sigma - VP)}{W(V+W)} + \frac{\sigma.dP}{P.dV}\right] \times \left[V^{1/3} + (V+W)^{1/3}\right]}{\left[V^{-2/3} + (V+W)^{-2/3}\right]}\right\}$$

where V is chamber · volume (ml), W is left ventricular wall
volume (0.943 ml/g ventricular weight) and P is end
diastolic pressure (dyne/cm2=7.5x10-4 mmHg). Myocardial
5   diastolic stiffness is calculated as the diastolic
stiffness constant (k, dimensionless), the slope of the
linear relation between E and σ (Mirsky and Parmley, 1973).
To assess contractile function, maximal +dP/dt was
calculated at a diastolic pressure of 5 mmHg.
10   The results are shown in Figure 10. All results
are given as mean ± SEM of at least 6 experiments. The
negative log EC50 of the increase in either force of
contraction in mN or rate of contraction in beats/min was
determined from the concentration giving half-maximal
15   responses in individual concentration-response curves.
Renal function results were corrected for kidney wet weight
measured at the end of the experiment. These results were
analysed by two-way analysis of variance followed by the
Duncan test to determine differences between treatment
20   groups and by paired or unpaired t-tests as appropriate;
p<0.05 was considered significant.
          At the end of the experiment, the atria and right
ventricle were dissected away and the weight of the left
ventricle plus septum was recorded.
25   L-NAME treatment markedly increased the diastolic
stiffness constant of the ventricles when compared to
controls. Developed pressure and contractility were not
altered by L-NAME treatment. C5a receptor antagonist
treatment prevented the increased diastolic stiffness
30   constant of L-NAME rats without altering contractility or
developed pressure. These results are presented in Figures
10 and 11.

d)   Isolated cardiac muscles and thoracic aortic rings
35   The heart is removed under anaesthesia. The right
atria and papillary muscles from the left ventricle are

removed and suspend ed in organ baths a a resting tension
of 5-10 mN adjusted . to give the maxima twitch response.
Tissues are bathed in a modified Tyrode's solution,
containing the following concentrations of salts in mM:

5   NaCl 136.9, KCl 5.4, MgCl$_2$ 1.05, CaCl$_2$ 1.8, NaHCO$_3$ 22.6,
NaH$_2$PO$_4$ 0.42, glucose 5.5, ascorbic acid 0.28, sodium
edetate 0.05, bubbled with 95% O$_2$/5%CO$_2$, and stimulated at
1Hz at 35°C as previously described (Brown et al, 1991a).
Cumulative concentration-response curves are measured for

10   noradrenaline and, following washout and re-equilibration,
for calcium chloride. At the end of the experiment,
papillary muscle dimensions are measured under the loading
conditions of the experiment; all tissues are blotted and
weighed.

·15   Thoracic aortic rings (approximately 4 mm in
length) are suspended with a resting tension of 10 mN
(Brown et al, 1991b) and contracted twice with isotonic KCl
(100 mM). The presence of endothelium is demonstrated by
addition of acetylcholine (1x10^{-5}M). Cumulative contraction

20   responses to noradrenaline are measured. Separate thoracic
aortic rings are perfused with 10% neutral buffered
formalin, embedded in wax and stained with haemotoxylin and
eosin. Image analysis using a Wild-Leitz MD30+ system is
used to calculate the wall area of the thoracic aorta.

25

Example 2   *Effect Of PMX53 on Bleomycin-induced*
                 Pulmonary Fibrosis

          Both acute and chronic diseases which induce
30   inflammation in the lung can lead to an irreversible
process characterized by pulmonary fibrosis. The effect of
PMX-53 on a rat model of pulmonary fibrosis was assessed,
using methods were adapted from Taylor et al (2002).
          Bleomycin is an antineoplastic agent which is a
35   well-known cause of pulmonary fibrosis in humans (Thrall et
al, 1978). Bleomycin-induced pulmonary fibrosis in rats is

a well-established model, which has a hort experimental
period and high suc cess rates. Bleomycn induces toxic
injury to Type I alveolar epithelial cells (AEC), which
causes release of TGF-β, PGE$_2$, granulocyte-macrophage colony
5   stimulating factor (GM-CSF), and insulin-like growth
factors etc. This induces a massive activation of
inflammatory cells such as PMNs, macrophages and
mesenchymal cells such as fibroblasts, which contribute to
an overaggressive repair process, leading to fibrosis in
10   the lung. PMX53 is a C5a receptor antagonist, which
effectively inhibits the infiltration and the activation of
inflammatory cells, such as PMNs, monocytes, macrophages,
and therefore reduces the release of reactive oxygen
species and inflammatory mediators such as IL-1 and PLA$_2$.
15   As a result, local tissue damage is prevented by a
reduction of release of several factors, such as
leukotrienes, and prostaglandins. We investigated whether
PMX53 antagonists had any inhibitory effect on bleomycin-
induced pulmonary fibrosis.
20   Male Wistar rats, 6 weeks of age, were used. The
rats were divided into 5 groups:
            Group 1: bleomycin instillation only (n=9)
            Group 2: saline instillation only (n=3)
            Group 3: PMX53 at a dose rate of 10mg/kg in 200µl
25   water p.o. (gavage daily) and bleomycin instillation (n=9)
            Group 4: PMX53 (dose as for Group 3) p.o. and
saline instillation (n=3).
            Group 5: Untreated rats maintained in the same
environment as the other groups (n=3).
30   Drug-treated rats were given drug for 3 days
before bleomycin administration.
            One intra-tracheal instillation of bleomycin at a
dose of 0.5mg/100g (0.7U/100g) in 200µl of saline was
performed on Day 1, as described by Taylor et al., (2002).
35   Rats were anaesthetized by inhalation of 5% or less
halothane via a vaporizer. After a local spray of

Xylocaine to prevent airway spasm, the rats were intubated and a slow injection of bleomycin or saline control was completed. The rats were then rotated gently for about 1-2 minutes to allow the solution to diffuse evenly into both

5   lungs (Christensen et al 2000). Rats were kept in the fume cupboard until totally recovered, and then monitored for up to 18 days. Body weight, food and water intake, and respiration were monitored daily.

Respiration was elevated as follows: Score 0,
10   normal respiration; Score 1, increased rate of breathing; and Score 2, mouth open respiration. Rats were euthanased before the end of the experimental period, if they consistently lost more than 10% body weight for 48 hours, had Score 2 respiration or had Score 1 respiration for 48
15   hours.

At the end of this period the rats were killed by exsanguination under anaesthesia, so that the lungs were clear of blood. For each rat, the left lung was immediately frozen in liquid nitrogen and stored at -20^0C for
20   quantitative collagen analysis using hydroxyproline assay. The right lung was fully inflated and fixed with 10% formulated formalin by airway gravity fixation at a pressure of 30 cm water for 1 minute. Haematoxylin and eosin (H&E) and Picro Sirius Red (PR) staining for collagen
25   were performed to assess collagen deposition in the lung. For quantitation of collagen stained with PR, polarized light images were converted to grey scale, and the total number of white pixels (specific for collagen) per image was determined as a percentage of the total pixel area. The
30   procedure was applied to a total of four fields in the alveolar area and two fields in the peribronchial area and blood vessels per sample (Wang et al, 2000). The largest lobe of the right lung (from 4 lobes) in each rat was chosen. The data was analysed using the program "Sion
35   Image".

Hydroxyproline assay was performed by the method

of Christensen et al 1 (2000). Lung tisue was excised,
trimmed free of sur: rounding conductingairways, and
homogenized in 2mls saline. A 1ml aliquot of lung
homogenate was hydrolysed in 6N HCl (0.5ml of homogenate
5   and 0.5ml of 12N HCl) at 110^0C for 12 hours; 50μl aliquots
were added to 1ml of 14% chloramine T, 10% n-propanol, and
0.5M sodium acetate, pH 6.0. After 20min at 22^0C, 1ml of
Ehrlich's solution (1M p-dimethylaminobenzaldehyde in 70%
n-propanol and 20% perchloric acid) was added and allowed
10   to incubate at 65^0C for 15min. Absorbance was measured at
550nm, and the amount of hydroxyproline was determined
against a standard curve generated with the use of known
concentrations of reagent-grade hydroxyproline.

        Data were compiled as the means ± SE in the study.
15   Tests of significance were obtained by ANOVA followed by
Student-Newman-Keuls post analysis. There were two stages
involved in the bleomycin-induced pulmonary fibrosis in rat
model.

20   1.   Acute lung inflammation:
        Intra-tracheal instillation of bleomycin induced
an acute lung inflammation in the rats, evident on Day 2 –
Day 3. Four of the rats from the drug-treated group and
four from the non-treated group were very ill, and had to
25   be euthanased after 7-9 days. The lungs appeared swollen,
with spreading white patchy lesions, as shown in Figures 12
and 13. The lung weight to body weight ratio was
significantly increased in bleomycin-treated rats,
regardless of whether the rats were drug-treated or non-
30   treated. The results are summarised in Table 1.

Table 1.

Lung weight and body weight in bleomycin-induced pulmonary
fibrosis (7-9 days)

Condition	Left lung weight (g)	Body weight (g)	Ratio x10^{-3}
Normal	0.507 ± 0.003	240.6 ± 4.667	1.9 ± 0.36
Bleomycin	1.004 ± 0.04	226 ± 8.083	4.47 ± 0.46**
Bleomycin + PMX53	0.974 ± 0.132	228 ± 7.583	4.25 ± 1.07**

**: P<0.001, n=3, compared to normal rats.

Under the microscope, numbers of inflammatory
cells, including PMNs, macrophages, lymphocytes etc. were
observed in the alveolar spaces, with massive leakage of
plasma and red blood cells; this is illustrated in Figure
13a. The size and number of type II AECs in the alveolar
spaces was clearly increased, as shown in Figure 13b, while
in normal lung, the type II AECs covered only 5 - 10% of
the surface area of the alveoli, as shown in Figure 14.
There was no significant difference in histology
between drug-treated and non-treated groups. Collagen
deposition in bleomycin instillation lungs showed a
significant increase compared to normal lungs (P<0.01,
n=3); saline instillation lungs (P<0.01, n=3); and saline
instillation with PMX53-treated lungs (P<0.01, n=3).
However, there was no significant difference between the
drug-treated group and non-treated group (P>0.01, n=4).
These results are summarised in Figure 15.

2. Pulmonary fibrosis
Eighteen days after intra-tracheal instillation of
bleomycin, the degree of oedema was reduced in bleomycin-
instilled lungs, and the lung/body weight ratio did not

show a significant · difference between ither the bleomycin
group and the non-b leomycin group, or etween the drug-
treated group and non-drug group (data not shown). As
illustrated in Figure 16, the inflammatory lesions in the
5 lung became smaller and less dense in most of the rats
compared with the acute inflammatory stage, whether or not
the rats had received drug treatment. There were still
numbers of inflammatory cells, many of which were alveolar
macrophages, and red blood cells in the lung lesions, as
10 shown in Figure 17a. The thickness of the alveolar walls
was increased, and there was some fibrinogen depositions in
alveolar septa in some of the lungs, as shown in Figure
17b. One drug-treated rat and one non-drug treated rat
still had some obvious lung inflammatory lesions mixed with
15 marked lung fibrosis lesions. It was difficult to assess
the quantity of collagen deposition in the lung tissues
from the H&E stained slides, because the amount of collagen
and the spread of the collagen varied in each individual
rat, and the number of the lesions in each lung was .
20 different. PR staining was more useful than H&E staining
for assessment of collagen deposition in the lungs, as
illustrated in Figures 21 to 30 and as summarised in Table
2.

25   Table 2.
       PR staining in bleomycin-induced pulmonary fibrosis (% of
                    the total pixel area, n=3-4)

Saline	Bleomycin	Bleo+PMX53
0.01	0.01	1.43
0.04	0.21	0.06
0.007	0.78	0.01
	1.73	0.77

30   However, for the same reasons it was not an

accurate measuremen t for comparison anlysis of the
collagen content. The hydroxyproline ssay results are
summarised in Figure 19. Bleomycin instillation
significantly increased hydroxyproline levels in the rat
5   lungs ($P<0.01$, n=3, compared to normal rats; $P<0.01$, n=3,
compared to saline instilled rats; $P<0.01$, n=3, compared to
saline instilled with drug treated rats). PMX53
significantly reduced the bleomycin-induced hydroxyproline
levels ($P<0.05$, n=4, compared to the rats with bleomycin
10   instillation).

The failure of PMX53 to inhibit the toxic lung
inflammation induced by bleomycin may indicate that the
bleomycin-induced toxic inflammation was initiated through
a different pathway or via a complicated inter-cellular
15   reaction, rather than by a simple activation of the
complement system. Type I AEC injury, type II AEC
proliferation, fibroblast proliferation, and release of
several cytokines, such as PGE_2, $TGF-\beta_1$, and GM-CSF, are
considered to play major roles in PF.
20.   After 18 days, the lungs with bleomycin
instillation showed some fibrosis, as demonstrated by the
significantly increased hydroxyproline levels and collagen
deposition as indicated by PR staining. We found that
PMX53 significantly reduced the hydroxyproline levels,
25   although this was difficult to confirm by histology or PR
staining. It is possible that 18 days is too early for the
histological changes to be evident, because most studies
demonstrated that the DNA and hydroxyproline changes occur
between 14-21 days after bleomycin instillation, while
30   histological evidence was present after 4 weeks.
Nevertheless, the significant reduction by PMX53
of bleomycin-induced hydroxyproline deposition indicates
that the activation of the C5a cascade may be involved in
the progression of fibrosis, although the role of C5a in
35   bleomycin-induced PF is not fully understood. It will be
apparent to the person skilled in the art that while the

invention has been described in some dtail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from
5   the scope of the inventive concept disclosed in this specification.

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10   following pages, and are incorporated herein by this reference.

36

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